

What is claimed is:

1. A probe set for detecting a genetic polymorphism in a nucleic acid sequence of a target nucleic acid suspected of containing said polymorphism, said probe set comprising:

a) a first flanking probe comprising

i) a sequence substantially complementary to a first portion of said nucleic acid sequence, and

ii) a first side chain,

b) at least one capture probe comprising

i) a sequence substantially complementary to a second portion of said nucleic acid sequence, said second portion comprising the location of said polymorphism, said second portion being adjacent to said first portion,

ii) a second side chain substantially complementary to said first side chain, and

iii) a third side chain and

c) a second flanking probe comprising

i) a sequence substantially complementary to a third portion of said target nucleic acid sequence, said third portion being adjacent to said second portion, and

ii) a fourth side chain substantially complementary to said third side chain,

wherein said first and second side chains and said third and fourth side chains non-covalently bind to form first and second stems, respectively, upon base pairing of said probes to said target nucleic acid sequence, and wherein at least one of said first and second side chains and at least one of said third and fourth side chains comprises an activatable crosslinking group, which upon activation forms a covalent cross-link with the other side chain comprising said stem, and wherein at least one of said first and second flanking probes comprises, in the sequence which is substantially complementary to its respective portion of said nucleic acid sequence, an activatable crosslinking group which upon activation forms a covalent crosslink with said respective portion.

2. A probe set according to claim 1 comprising an additional capture probe which is complementary to the normal nucleic acid sequence of said second portion lacking said polymorphism.

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3. A probe set according to claim 2 additionally comprising a reporter moiety comprising a detectable label.

4. A probe set according to claim 2, wherein said crosslinking group is photoactivable.

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5. A probe set according to claim 4, wherein said photoactivatable group is a coumarin, furocoumarin or psoralen.

6. A probe set according to claim 5 wherein the crosslinking compound is selected from the group consisting of coumarin, coumarin derivatives, O-(7-coumarinyl) glycerol; psoralen, psoralen derivatives, 8-methoxypsoralen, 5-methoxypsoralen; cis-benzodipyrene, cis-benzodipyrene derivatives; trans-benzodipyrene, trans-benzodipyrene derivatives; and compounds containing fused coumarin-cinnoline ring systems.

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7. A probe set according to claim 6 wherein said photoactivable group is O-(7-coumarinyl) glycerol.

8. A probe set according to claim 4 wherein the polymorphism is a single nucleotide polymorphism.

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9. A probe set according to claim 4 wherein the capture probes are biotinylated.

10. A probe set according to claim 8 comprising a fluoresceinated reporter molecule hybridizable to said target sequence.

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11. The probe set of claim 4 wherein said polymorphism is a point mutation (G1691A) in the Factor V gene.

5 12. The probe set of claim 11 comprising

a first capture probe having the sequence of SEQ ID NO: 1

a second capture probe having the sequence of SEQ ID NO: 2

a first flanking probe having the sequence of SEQ ID NO: 3

a second flanking probe having the sequence of SEQ ID NO: 4

10 and a third flanking probe having the sequence of SEQ ID NO: 5.

13. The probe set of claim 12 comprising a fluoresceinated reporter molecule hybridizable to said target sequence.

15 14. The probe set according to claim 13 comprising a reporter probe selected from the group consisting of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO: 12.

20 15. The probe set of claim 4 wherein said polymorphism is a point mutation (C187G) in the HFE gene.

16. The probe set of claim 15 comprising

a first capture probe having the sequence of SEQ ID NO: 14

a second capture probe having the sequence of SEQ ID NO: 15

25 a first flanking probe having the sequence of SEQ ID NO: 16

and a second flanking probe having the sequence of SEQ ID NO: 17.

17. The probe set of claim 16 comprising a fluoresceinated reporter molecule hybridizable to said target sequence.

18. The probe set according to claim 17 comprising a reporter probe selected from the group consisting of SEQ ID NO.: 18, SEQ ID NO.: 19, SEQ ID NO.: 20, SEQ ID NO.: 21, SEQ ID NO.: 22, SEQ ID NO.: 23, and SEQ ID NO.: 24.

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19. The probe set of claim 4 wherein said polymorphism is a point mutation (G845A) in the HFE gene.

20. The probe set of claim 19 comprising

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- a first capture probe having the sequence of SEQ ID NO: 25
- a second capture probe having the sequence of SEQ ID NO: 26
- a first flanking probe having the sequence of SEQ ID NO: 27
- and a second flanking probe having the sequence of SEQ ID NO: 28.

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21. The probe set according to claim 20 comprising a fluoresceinated reporter probe hybridizable to said target sequence.

22. The probe set according to claim 21 comprising a reporter probe selected from the group consisting of SEQ ID NO.:29, SEQ ID NO.:30, SEQ ID NO.:31, SEQ ID NO.:32, SEQ ID NO.:33, SEQ ID NO.:34, SEQ ID NO.:35, SEQ ID NO.:36, SEQ ID NO.:37, SEQ ID NO.:38, and SEQ ID NO.:39.

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23. A method for detecting a genetic polymorphism in a nucleic acid sequence of a target nucleic acid suspected of containing said polymorphism, said method comprising:

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combining, in a hybridizing medium, a nucleic acid sample comprising said target and a plurality of probes under hybridizing conditions for a time sufficient for said target and said probes to hybridize, wherein said plurality of probes comprises

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a) a first flanking probe comprising

i) a sequence substantially complementary to a first portion of said nucleic acid sequence, and

ii) a first side chain,

b) at least one capture probe comprising

i) a sequence substantially complementary to a second portion of said nucleic acid sequence, said second portion comprising the location of said polymorphism, said second portion being adjacent to said first portion,

ii) a second side chain substantially complementary to said first side chain, and

iii) a third side chain and

c) a second flanking probe comprising

i) a sequence substantially complementary to a third portion of said target nucleic acid sequence, said third portion being adjacent to said second portion, and

ii) a fourth side chain substantially complementary to said third side chain, wherein said first and second side chains and said third and fourth side chains non-covalently bind to form first and second stems, respectively, upon base pairing of said probes to said target nucleic acid sequence, and wherein at least one of said first and second side chains and at least one of said third and fourth side chains comprises an activatable crosslinking group, which upon activation forms a covalent cross-link with the other side chain comprising said stem, and wherein at least one of said first and second flanking probes comprises, in the sequence which is substantially complementary to its respective portion of said nucleic acid sequence, an activatable crosslinking group which upon activation forms a covalent crosslink with said respective portion

comparing the degree of hybridization of said capture probe to said sequence portion containing said polymorphism to the hybridization of a capture probe to said target sequence lacking said polymorphism whereby the polymorphism is determined.

24. A method according to claim 23 comprising an additional capture probe which is complementary to the normal nucleic acid sequence of said second portion lacking said polymorphism.

25. A method according to claim 24 additionally comprising a reporter moiety comprising a detectable label.

26. A method according to claim 25, wherein said crosslinking group is photoactivable.

27. A method according to claim 26, wherein said photoactivatable group is a coumarin, furocoumarin or psoralen.

28. A method according to claim 27 wherein the crosslinking compound is selected from the group consisting of coumarin, coumarin derivatives, O-(7-coumarinyl) glycerol; psoralen, psoralen derivatives, 8-methoxypsoralen, 5-methoxypsoralen; cis-benzodipyrene, cis-benzodipyrene derivatives; trans-benzodipyrene, trans-benzodipyrene derivatives; and compounds containing fused coumarin-cinnoline ring systems.

29. A method according to claim 28 wherein said photoactivable group is O-(7-coumarinyl) glycerol.

30. A method according to claim 26 wherein the polymorphism is a single nucleotide polymorphism.

31. A method according to claim 26 wherein the capture probes are biotinylated.

32. A method according to claim 30 comprising a fluoresceinated reporter molecule  
5 hybridizable to said target sequence.

33. The method of claim 26 wherein said polymorphism is a point mutation (G1691A) in  
the Factor V gene.

10 34. The method of claim 33 comprising

a first capture probe having the sequence of SEQ ID NO: 1

a second capture probe having the sequence of SEQ ID NO: 2

a first flanking probe having the sequence of SEQ ID NO: 3

a second flanking probe having the sequence of SEQ ID NO: 4

15 and a third flanking probe having the sequence of SEQ ID NO: 5

35. The method of claim 34 comprising a fluoresceinated reporter molecule hybridizable  
to said target sequence.

20 36. The method according to claim 35 comprising a reporter probe selected from the  
group consisting of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9,  
SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO: 12.

25 37. The method of claim 26 wherein said polymorphism is a point mutation (C187G) in  
the HFE gene.

38. The method of claim 37 comprising

a first capture probe having the sequence of SEQ ID NO: 14

a second capture probe having the sequence of SEQ ID NO: 15

30 a first flanking probe having the sequence of SEQ ID NO: 16

and a second flanking probe having the sequence of SEQ ID NO: 17.

39. The method of claim 38 comprising a fluoresceinated reporter molecule hybridizable to said target sequence.

40. The method according to claim 39 comprising a reporter probe selected from the group consisting of SEQ ID NO.: 18, SEQ ID NO.: 19, SEQ ID NO.: 20, SEQ ID NO.: 21, SEQ ID NO.: 22, SEQ ID NO.: 23, and SEQ ID NO.: 24.

41. The method of claim 26 wherein said polymorphism is a point mutation (G845A) in the HFE gene.

42. The method of claim 41 comprising  
a first capture probe having the sequence of SEQ ID NO: 25  
a second capture probe having the sequence of SEQ ID NO: 26  
a first flanking probe having the sequence of SEQ ID NO: 27  
and a second flanking probe having the sequence of SEQ ID NO: 28

43. The method according to claim 42 comprising a fluoresceinated reporter probe hybridizable to said target sequence.

44. The method according to claim 43 comprising a reporter probe selected from the group consisting of SEQ ID NO.:29, SEQ ID NO.:30, SEQ ID NO.:31, SEQ ID NO.:32, SEQ ID NO.:33, SEQ ID NO.:34, SEQ ID NO.:35, SEQ ID NO.:36, SEQ ID NO.:37, SEQ ID NO.:38, and SEQ ID NO.:39.

45. The method of claim 23 wherein the probes are comprised of ribonucleic acid.

46. The method of claim 23 wherein the probes are comprised of deoxyribonucleic acid.